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Audiogram of a stranded Blainville's beaked whale (*Mesoplodon densirostris*)

measured using auditory evoked potentials

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KEYWORDS

Blainville's beaked whale, hearing, marine mammals, acoustics, auditory evoked potentials

ABBREVIATIONS

ABR: Auditory Brainstem Response
AEP Auditory evoked potentials
EFR Envelope following response
SAM: sinusoidally amplitude modulated
HCRF: Hawaii Cetacean Rehabilitation Center
FFT: Fast Fourier Transform
r.m.s : root-mean-square
FM: frequency modulated
LFAS : low frequency active sonar
MFAS: Mid frequency active sonar

ABSTRACT

Quantifying and understanding the impact of anthropogenic sound on marine mammals has been the focus of many researchers both in laboratory settings as well as in the field. This study presents the audiogram of a sub-adult Blainville's beaked whale that stranded in Hawaii. The hearing measurements were conducted using the non-invasive auditory brainstem response technique and a total of 11 sinusoidally amplitude modulated tones were tested ranging from 5.6 to 160 kHz. The audiogram data indicated that the region of best hearing was found between 40 and 50 kHz with thresholds below 50 dB. These results match the frequency range obtained from the hearing measurements of a Gervais' beaked whale previously tested using contact acoustic stimulation and emphasize the importance of obtaining rapid hearing measurements on live stranded animals to improve the understanding of poorly known species.

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INTRODUCTION

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Beaked whales are deep diving and cryptic animals belonging to the Ziphiidae family containing an unusually large number of species (21) for odontocetes. Most beaked whale species are characterized by an "pronounced beak, relatively small dorsal fin set far back on the body, small flippers that fit into depressions on the sides," the reduction in the number of erupted teeth and the presence of converging throat grooves (Jefferson, Webber and Pitman, 2008). These animals are rarely encountered in the wild and very little is known about their ecology, behavior and life history. Blainville's beaked whales *Mesoplodon densirostris* (Blainville, 1817) have the widest distribution within the *Mesoplodon* genus; they inhabit temperate and tropical areas and are found primarily in deep waters. The animals are usually found in waters with depths around 1000m with a steep bathymetry where they are assumed to forage (Baird et al., 2006) on squid and deep water fish (Mead, 1989; Santos et al., 2001). Individuals are usually brownish to dark grey dorsally and lighter ventrally; they can reach up to 4.7 meters in length and weigh over 1000 kg (Jefferson, Webber & Pitman, 2008). Most animals exhibit oval white scarring presumably from cookie cutter shark bites (Fig. 6).

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Photo identification and satellite tagging have provided some information about habitat use and site fidelity in Blainville's beaked whales. In the main Hawaiian Islands, this species may exhibit strong site fidelity and the population appears to be island-associated (Schorr et al., 2009; McSweeney et al., 2007). The use of acoustic recording suction cup tags has provided valuable information about the foraging behavior of these deep diving cetaceans. They do not initiate echolocating at depths shallower than 200m (Johnson et al., 2004). Their foraging dives have a mean duration of 47min and are at an average depth of 840m. These long dives are usually followed by series of shallow dives with no apparent foraging attempts. These shallow dives are hypothesized to help the animals recover from the long foraging bouts (Tyack et al., 2006).

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The properties of the far field echolocation clicks were obtained when a conspecific fortuitously echolocated on an acoustically-tagged animal. Blainville's beaked whale echolocation signals have been compared to those produced by a variety of bat species. They produce two distinct echolocation signals (Johnson et al., 2006; Madsen et al., 2005). During the search phase, they emit a long click, approximately 271 μ s in duration, with a frequency modulated upswEEP component. Most of the acoustic energy is found between 26 and 51 kHz (Johnson et al., 2006). The second type of echolocation signal is a terminal buzz (Griffin, 1958) where the inter-click-interval decreases towards the end of a click train as the animal presumably closes in on its prey. Similar to other odontocete clicks, the *Mesoplodon*

terminal buzz clicks have no frequency modulated component, a broader bandwidth and a shorter duration (105 μ s).

Much of the interest in beaked whales in recent years has been generated by their association with navy sonar exercises and subsequent strandings. Several mass strandings of beaked whales have occurred following the broadcasting of low and mid frequency military sonars (Frantzis, 1998; reviewed by Cox et al., 2006). While the underlying causes of these strandings remain unknown, some hypotheses have been presented such as the formation of gas bubbles from direct ensonification or complications due to alterations in the diving behavior (Houser et al., 2001; Jepson et al., 2003; Fernandez et al., 2005; Rommel et al., 2006; Hooker et al., 2009). Unfortunately because beaked whales are poorly understood, the underlying causes of their mass stranding remain hypothetical and call for more in-depth research on their behavior and physiology. Regardless of the cause of these strandings, the animal's ear is the most sensitive detector of acoustic energy. It is extremely unlikely that an animal will suffer injury from a sound that it cannot hear.

It is important to understand the effects of anthropogenic sounds on marine mammals (Tyack, 2008; Nowacek, 2007), and tremendous efforts have been invested into understanding and quantifying the human contribution on ocean noise, designing integrative models to predict ocean noise and into conducting research on marine species that are likely to be at risk (National Research Council, 2003; 2005). Studies looking at the effects of acute and chronic sound exposure both in the short term (Miller et al., 2000; Romano et al., 2004; Talpalar and Grossman, 2005; Di Iorio and Clark, 2010) as well as in the long term (Tyack, 2008) have shown that anthropogenic sound is likely to affect marine mammal populations. Southall et al. (2007) noted that carefully controlled studies of hearing sensitivity, particularly for high-priority species such as beaked whales, were a "critical information need". As part of this effort, techniques for examining the basic hearing of marine mammals have been developed over the past 20 years and to this date, the audiogram of only one other beaked whale species, the Gervais' beaked whale *Mesoplodon europaeus* has been obtained (Cook et al., 2006; Finneran et al., 2009). The use of envelope following response (EFR) auditory evoked potential (AEP) technique provides a unique platform to obtain audiograms rapidly with untrained animals (Supin et al., 2001; Nachtigall et al., 2007). The AEP technique yields results comparable to more traditional behavioral audiograms (Yuen et al., 2005; Finneran & Houser, 2006) allowing hearing measurements of species found in non-laboratory settings to be obtained such as oceanarium animals (Szymanski et al., 1999) as well as temporarily caught odontocetes (Nachtigall et al., 2008) and long-term rehabilitated odontocetes (Pacini et al., 2010).

A single Blainville's beaked whale stranded in Kihei, Maui on August 16th 2010 and was transported to the Hawaii Cetacean Rehabilitation Center in Hilo, Hawaii (HCRF). Hearing measurements were collected for frequencies between 5.6 and 160 kHz within the first two days of the animal's rehabilitation. The results provide the first basic hearing measurements for the Blainville's beaked whale.

METHODS

Subject

The male sub-adult *Mesoplodon densirostris* was found stranded on the morning of August 16th, 2010 near Kihei on the island of Maui. The animal was observed milling in very shallow waters for several hours. On physical examination, the animal appeared weak and dehydrated. Initial diagnostics indicated severe immune compromise and renal insufficiency. The animal was given mineral and electrolyte injections and transported via a Coast Guard flight to the University of Hawaii Hilo Cetacean Rehabilitation Facility (HCRF). The whale was 3.5 meters long and weighed approximately 800 kg. Once at the rehabilitation center, he was tube-fed every hour with a mixture of water, electrolyte solution, medications and ground squid. Acoustic testing was selected as a 'non-invasive' ancillary diagnostic test to aid in the determination of the animal's medical problem and prognosis for rehabilitation.

Hearing measurements were collected periodically while it was undergoing medical treatments from August 16th until August 18th. The animal was lightly restrained during the hearing measurements while monitoring its respiratory rate. Overall behavior indicated that there was no aversive reaction to the presentation of sound or the hearing measurements.

Intensive care of rehabilitation efforts continued with the animal. After several days in the hospital facility, the whale developed severe gastrointestinal hemorrhage and displayed signs consistent with respiratory disease. It died on August 29th, 2010.

Tank and Background noise measurements

The animal was housed in the covered oval rehabilitation pool of the Hilo rehabilitation center. The oval pool was 9.8 m long, 7.3m wide and 1.5 m deep. Water pumps and filters were turned off during hearing measurements to limit masking background noise. The background noise was measured using a Reson TC-4040 hydrophone (- 206 dB dr 1V/mPa; Slangerup, Denmark) and recorded as one minute files with a Microtrack II 2 channel digital recorder (M-Audio, Irwindale, CA, USA) with a 96 kHz sampling rate. Alienated signals were compensated by having a channel with no gain and the other

channel with a 15 dB gain. Ten one second files were extracted using Adobe Audition 3.0, analyzed, fast Fourier transformed using a 1024 point FFT and averaged with a customized Matlab algorithm.

Acoustic stimulus

The AEP measurement system used during the hearing measurements was similar to the equipment presented by Taylor et al (2007) and used in the Mooney et al. (2008), Nachtigall et al. (2008) and Pacini et al.(2010) studies. During the hearing measurements, the animal was held at the surface in the middle of the rehabilitation pool and a projecting transducer was positioned 1m away from the animal's head (Fig X) at a 30cm depth marked by a colored tape placed on the transducer cord. Three latex suction cups containing Grass 10 mm gold EEG electrodes (West Warwick, RI, USA) were positioned on the head and back of the animal to collect electrophysiological records.

The acoustic stimuli consisted of sinusoidally amplitude modulated tone bursts that were digitally generated using a customized Labview program and a National Instrument PCMIA-6062 E DAQ card (Austin, TX, USA) implemented in a laptop. The tone bursts were 19ms in duration and followed by 30 ms of silence yielding a 20 ms^{-1} presentation rate. The tones were modulated at a 1000 Hz rate based on the modulation rate transfer function obtained prior to the audiogram measurements and previous results obtained with beaked whales (Finneran et al., 2009). For frequencies lower than 50 kHz a 256 kHz update rate was used and increased to 512 kHz for frequencies between 50 kHz and 100 kHz and 800 kHz for frequencies above 100 kHz. Peak-to-peak voltages (V_{p-p}) were measured using a Tektronix TPS 2014 oscilloscope (Beaverton, OR, USA) and then converted to peak equivalent root mean square (r.m.s.) voltages by subtracting 15 dB. SPLs were varied in 1 to 10 dB steps using a Hewlett-Packard P-350D attenuator (Palo Alto, CA, USA). These r.m.s. voltages were then used to calculate the sound pressure level (SPL) for each frequency. Two hydrophones were used to present the acoustic stimulus: an ITC-1032 (Santa Barbara, CA, USA) for the low frequencies between 5.6 and 40 kHz and a Reson TC-4013 for frequencies above 50 kHz. A total of 11 frequencies were tested from 5.6 to 160 kHz.

Electrophysiology measurements

Three Grass (West Warwick, RI, USA) 10mm gold EEG electrodes embedded in latex suction cups were positioned on the animal. The active electrode was positioned over the brain 10 cm behind the blow hole and 3-4cm off to the right side of the animal's head, the reference on the back of the subject while the ground electrode was positioned laterally on the animal's dorsal fin (Fig. 6). The electrophysiological signal was amplified 10,000 times and filtered from 300 to 3000 Hz using a Grass

CP-511 bio-amplifier (West Warwick, RI, USA). Additional by-pass filtering was obtained with a Krohn-Hite 3384 filter (Brockton, MA, USA). The same laptop computer and card were used to present the acoustic stimulus and to digitize the electrophysiological response using a 16 kHz sampling rate. A full record - or trial - took approximately 90 sec and consisted of collecting and averaging 1000 responses, each 26ms long and triggered with the acoustic stimulus.

Data analysis

The complete audiogram was obtained over the course of 48 hours. The data collection effort was divided into sessions of 20 minutes to avoid interfering with other medical and diagnostic tests, feeding and resting periods.

Each threshold was calculated using at least 7 trials or records for each frequency. The level of the first sound for each frequency was chosen based on previous audiograms and was 15-20 dB above the published thresholds for other odontocetes (Pacini et al. 2010; Finneran et al. 2009; Nachtigall et al., 2008, Johnson, 1967) The SPL was then varied in 5-10 dB steps until the evoked potential response was low enough so as to not be discernable from the ambient biological noise for at least two trials. SAM tone bursts are known to generate a rhythmic response known as an EFR (Supin et al., 2001; Nachtigall et al., 2007). At each SPL, a 16 ms window of the EFR was analyzed using 256 point fast Fourier Transform (FFT). The peak response at 1000 Hz on the obtained frequency spectrum was used to estimate the animal's response to the acoustic stimulus. For each frequency, the peak responses at 1000 Hz were then plotted against the stimulus SPL and a linear regression addressing the data points was used to evaluate the hypothetical zero value used to predict the threshold. The ABR technique does not yield absolute thresholds due to the inherent biological noise, but previous work has shown that the results are comparable to behavioral audiograms. (Yuen et al., 2005)

RESULTS

The rehabilitation pool at HRCF provided a relatively quiet environment for the hearing measurements because most of the energy was below 1 kHz. Above 1 kHz, most of the ambient noise was below 60 dB and below the sensitivity of the recording equipment. All hearing data were collected with the pumps and filters turned off providing limited masking effects. The background noise is plotted in Fig. 1B.

The EFR had a delay of 4-6 ms which corresponded to the latency of the neurophysiological response. Overall the EFR was similar to measurements obtained with other odontocete species

(Thomas et al., 1988; Nachtigall et al., 2004; Szymanski et al., 1999). With a SPL well above the threshold level, the EFR formed a complete rhythmic response which decreased with the SPL. As the SPL approached the threshold level, the rhythmic EFR disappeared in the inherent biological noise. Fig X shows the EFR to a SAM tone at 150 kHz. At 135 dB, the EFR was fully formed and closely followed the envelope of the acoustic stimulus. The EFR decreased in magnitude as the SPL of the acoustic stimulus decreased. At 115 dB, the rhythmic pattern was indiscernible from the background noise. The linear regression for that specific frequency yielded a 116.0 dB threshold.

The audiogram had the common U-shape found in mammalian species and the hearing range was similar to typical odontocete audiograms (Johnson, 1967, Houser et al., 2008, Thomas et al., 1988) with a steep slope in the high frequency region and a more leveled slope in the low frequency range. The area of best hearing was found between 40 and 50 kHz forming a broad notch in the audiogram. The best hearing was found at 50 kHz with a 48.9 dB threshold. Past 50 kHz, the slope of the threshold curve increased rapidly and the ranges of poorest hearing were found at both ends of the frequency spectrum with thresholds of 79 dB for 5.6 kHz and 116 dB for 160 kHz. Overall, the low ambient noise of the pool (Fig 1B) provided a quiet environment and masking effects were low, yielding threshold measurements with comparatively low values down to the 50 dB ranges in this relatively quiet environment (Au et al., 2002).

DISCUSSION

The audiogram of this *M. densirostris* is similar to audiograms of other odontocete species with a typical U-shape curve and good hearing in the human ultrasonic range. Thresholds below 50 dB indicate that the environment was likely suitable for hearing measurements and that masking effects were negligible. The high frequency cut-off of the animal's hearing is relatively low compared to small odontocetes that have an area of best hearing around 40-50 kHz. In young bottlenose dolphins, the best hearing usually lies around 80 kHz (Johnson, 1967) and up to 120-140 kHz for harbor porpoises and white-beaked dolphins with an area of best hearing between 100-140 kHz and 45-128 kHz respectively (Nachtigall et al., 2008; Kastelein et al., 2002). The audiogram of *M. densirostris* was similar to larger odontocetes audiogram such as the Gervais' beaked whale *Mesoplodon europaeus* (Finneran et al., 2009) the long-finned pilot whale *Globicephala melas* (Pacini et al., 2010) and the killer whale *Orcinus orca* (Szymanski et al., 1999) indicating that size might influence not only the sound production mechanisms (Wang et al., 1995) but also the hearing range of the animals, a pattern well documented in terrestrial mammals (Heffner and Heffner, 1983).

230 In comparison to the hearing measurements of Gervais' beaked whales (Cook et al., 2006;
231 Finneran et al., 2009), the audiogram obtained here is similar in shape but very different in threshold
232 values. Most of the thresholds were at least 20 dB more sensitive than the Gervais' beaked whale
233 thresholds. In that particular study, the acoustic stimulus was presented via a contact hydrophone
234 positioned underwater on the panbone region of the lower jaw. That technique has been shown to
235 produce comparable results to far-field audiograms in bottlenose dolphins (Finneran et al., 2006) and
236 was preferred by the investigators to limit the effects of the animal's head movements on the threshold
237 calculations. The authors, however, noted that this underwater jawphone method had not been
238 calibrated for beaked whale species and that the thresholds values should be interpreted carefully as
239 they were extrapolated from calibrations obtained with *Tursiops truncatus*.

240 Similar to the present hearing measurement of *M. densirostris*, the range of lowest thresholds
241 or most sensitive hearing with *M. europaeus* was between 40-60 kHz. Additionally, while no responses
242 could be detected above 80 kHz for the *M. europaeus* (Finneran et al., 2009), our work with no
243 jawphone and in-water free-field sound presentation yielded thresholds in the 100 dB range for
244 frequencies between 80 and 160 kHz for *M. densirostris*. These results may indicate – as suspected by
245 Finneran et al. (2009) - that the calibration of the jawphone acoustic stimulus for a new species might
246 have represented a difficulty. Alternatively, the Gervais beaked whale simply did not hear overall as well
247 as the Blainville's beaked whale in this study. Variability in threshold levels between individuals, even
248 within a species is not uncommon (Finneran and Houser, 2006).

249 Many factors are known to influence hearing, from variations across individuals (Houser et al.,
250 2008; Popov et al., 2007) to environmental factors such as acoustic ambient noise (Kei et al., 2008)).
251 Whether the two complete beaked whale audiograms are representative of beaked whale hearing or
252 just ends of the spectrum of individual variation can only be determined as more audiograms become
253 available. In this study, the animal was a sub-adult male whose teeth had not yet erupted. In
254 comparison, the *M. europaeus* was a mature adult of unknown age and hearing loss could not be ruled
255 out. Younger animals tend to hear better and presbycusis or hearing loss due to age has been
256 documented in marine mammals and is likely to occur in the high frequency range (Ridgway and Carder,
257 1996; Demeester et al., 2009; Houser et al., 2008; Kloepper et al., 2010). The subject in the present
258 study was not full grown and presbycusis does not appear to be a potential cause of the observed
259 limited high frequency hearing. In addition, the *M. densirostris* was not administered any ototoxic
260 medicine during its rehabilitation.

Acoustic tagging has provided information on the echolocation behavior of Blainville's beaked whales. In general, beaked whales are deep divers, and so far, echolocation has only been detected when individuals are below a depth of 200m (Tyack et al., 2006; Johnson et al., 2004). The clicks used during the searching phase of a foraging bout differ from most odontocete clicks. The signals are longer in duration and are characterized by a FM upsweep with a -10 dB bandwidth between 26 and 51 kHz (Johnson et al., 2006). The buzz phase clicks used in the final approach before prey capture have a broader bandwidth and are very similar to other odontocetes' clicks. FM bats appear to use a similar method of prey detection and capture (Madsen et al., 2005) and their best hearing usually lies within the range of echolocation frequencies of their signals (Neuweiler, 1984). Some species have even been shown to possess a cochlear acoustic fovea centered on the area of their echolocation clicks (Schuller et al. Pollack, 1979).

The audiogram collected in this study - combined with the acoustic data obtained by Johnson et al. (2006) - indicates that the area of best hearing partially overlaps with the frequency spectrum of the FM signals used by *M. densirostris*. Other odontocetes such as the bottlenose dolphins use broadband echolocation clicks and are thought to rely on an energy detector receiver model using these short pulsed signals (Au, 1993). Beaked whale FM clicks resemble the FM signals used by bats, which are believed to rely on a matched filter receiver model where the animal innately compares the received echo to the outgoing click to obtain ranging information. Why and whether beaked whales would rely on a different technique from other odontocetes remains unknown and might be related to their unique life history. Johnson et al. (2006) hypothesized that the use of FM signals during the search phase might improve the detection and discrimination of specific prey in a scattered environment and thus "maximizing the net energy return of foraging during long breath-hold dives." (Johnson et al., 2006) If *M. densirostris* relies on a different echolocation strategy to locate and identify their prey and use "prey-specific signatures in the returning echoes" (Madsen et al., 2005), extremely sensitive hearing in the frequency range of the FM clicks would represent a definite advantage to cross correlate the returning echo to the emitted signal.

While acoustic tagging research has begun to provide a more comprehensive picture of beaked whales' ecology and behavior, these species remain amongst the most cryptic marine mammals. Some species have been only identified only within the last 10 years and have never been observed alive (Dalebout et al., 2002; Reyes et al., 1991). Most of the knowledge about this beaked whale has been obtained through strandings. In recent years, special interest has arisen after multiple unusual mass strandings have been linked to military exercises (reviewed in Cox et al., 2006; Rommel et al., 2006;

Nowacek et al., 2007). In 1996, the mass stranding of 13 Cuvier's beaked whales (*Ziphius cavirostris*) was found to coincide with NATO activities using LFAS (Frantzis, 1998). In 2000, 17 cetaceans including a single Blainville's beaked whale stranded in the Bahamas during a naval exercise and the interim report indicated that the use of the mid frequency active sonar was the 'most plausible cause' of this mass stranding (US Department of Commerce and US Department of the Navy, 2001). In 2002 during the Neo-Tapon international naval exercise, another stranding involving 14 beaked whales including 3 Blainville's beaked whales occurred and was also linked to the use of MFAS. Some common trends arise from these strandings, including bathymetry profile, sound levels used and the strong links both temporally and geographically to naval active sonars (Cox et al, 2006). MFAS uses frequencies between 1-10 kHz. The Blainville's beaked whale hearing threshold at 5.6 kHz indicated that the animal was able to detect this frequency at levels as low as 79 dB in a quiet environment.

At the time of the stranding of the animal examined in this study, no naval activity was reported. The animal stranded two weeks after the end of the biannual international Rim of the Pacific (RIMPAC) exercise. The animal died 13 days after it stranded in Maui. At the time of the writing of the manuscript, histopathology of the organ systems have not been completed. Based on the gross post mortem examination, organ cultures, viral serology and PCR testing, it has been hypothesized that the whale was likely suffering from a systemic viral infection that caused weakness and anorexia which ultimately led to dehydration and stranding. The immune compromised whale then developed a peracute bronchopneumonia with subsequent gastrointestinal ulcerations.

As any work obtained from a stranded animal, the present audiogram should be interpreted carefully. Strandings provide a rare opportunity to obtain physiological information about poorly known species. One of the main difficulties in studying marine mammals arises from the limited sample size available to researchers. As noted by Finneran et al. (2009), collecting data during a stranding event is not ideal; due to the unstable health of the animal and the limited time allocated to measurements, factors such as electrode placement and head movements must be carefully monitored and accounted for during the analysis, thus increasing potential errors in the measurements obtained.

This audiogram of a *M. densirostris* contributes to the ongoing effort to better understand the effects of noise on marine life. More importantly, these results provide valuable information about the hearing abilities of a species implicated in strandings related to naval exercises. In addition, they provide baseline data about the acoustic abilities of a poorly known but critically important species. This type of research – although not as controlled as laboratory settings – allows the scientific and management

communities to obtain crucial physiological information using non invasive techniques and provides a diagnostic tool to rapidly measure the hearing of wild animals.

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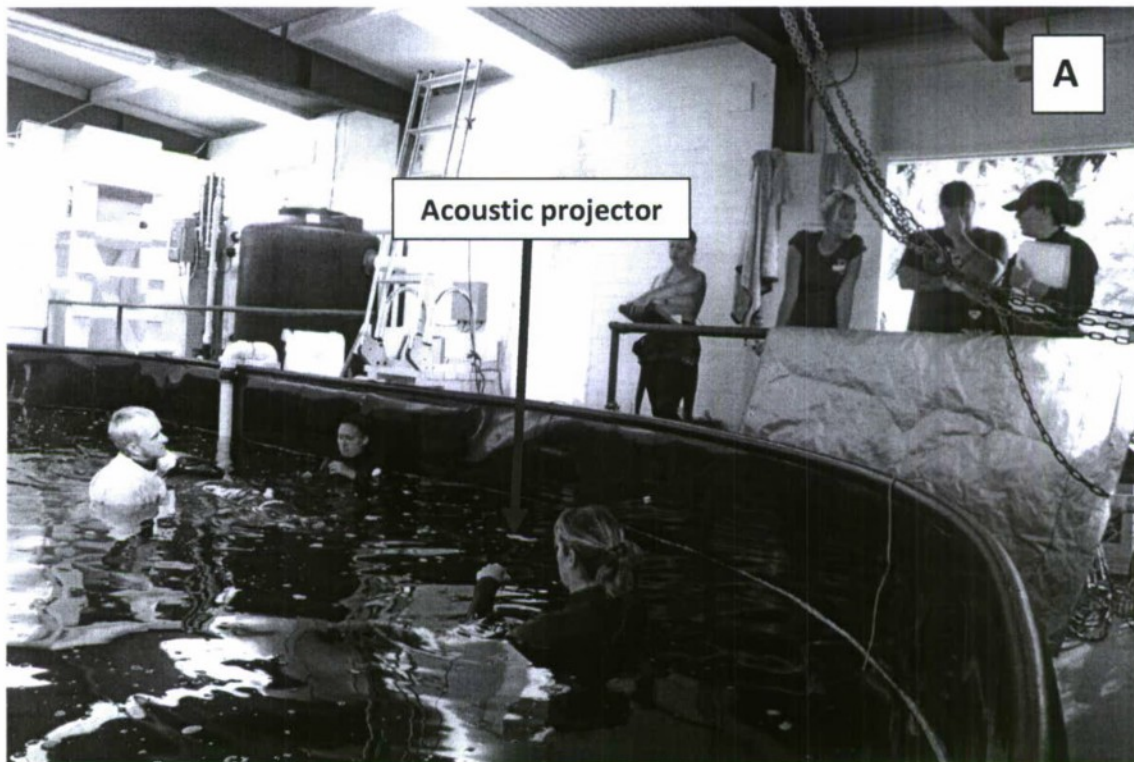
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514 Figure 1: (A) Rehabilitation pool where the hearing measurements were conducted. The water pumps
515 and filters are visible in the back and were turned off during the auditory tests. The projector was
516 positioned one meter away from the animal's head while the whale was lightly restrained (B) Tank
517 background noise was calculated using a 1024 point fast Fourier transform (FFT) and collected with a
518 Reson TC-4040 hydrophone with a 96 kHz sampling rate. Sound levels are expressed in $\text{dB } 1 \mu\text{Pa}^2\text{Hz}^{-1}$.
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